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Persistence of neutralizing antibody 30 – 35 years after immunization with 17D yellow fever vaccine

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Previous studies on the duration of antibody following vaccination with 17D yellow fever (17D YF) virus vaccine have indicated that immunity persists for at least 17 years and suggest that the vaccine may provide lifelong immunity. We studied sera obtained from 149 veterans of the Second World War, 30 – 35 years after military service during which YF vaccination was required for defined groups. A significantly high proportion of "vaccinated" subjects was found to be seropositive to 17D YF virus. The highest proportion of seropositive "vaccinated" veterans (97%) was among navy and air corps personnel, while only 60% of "vaccinated" army personnel and 19% of "unvaccinated" personnel were seropositive. This study suggests that (i) antibody to 17D YF virus, as measured by the plaque-reduction neutralization test (PRNT), persists for 30 years or more following administration of a potent vaccine; (ii) army personnel often had not received potent vaccine, even though their service history indicated that they should have been vaccinated; (iii) some personnel were vaccinated, although their service did not include vaccination-designated areas; and (iv) 88% of veterans with persistent PRNT antibody to 17D YF virus also had mouse-protective antibody against French neurotropic YF virus.

The only yellow fever (YF) vaccine licensed for use in the United States of America is a live, attenuated preparation of the 17D strain of YF virus (*1*). The duration of immunity following vaccination is not known, but international travellers entering or leaving a YF-endemic area are required to possess certification of YF immunization no more than 10 years previously (*2*). The Advisory Committee on Immunization Practices (ACIP) has stated that YF revaccination is not required "more often than every 10 years" (*1*), reflecting the suspicion that immunity may persist for more than 10 years (*3*).

To evaluate the persistence of antibody 30 – 35 years after vaccination with the 17D virus, we tested sera obtained in 1975-76 from US veterans of the Second World War who had been vaccinated before 1948. Extensive YF vaccination of US troops was undertaken during the Second World War, both to protect military personnel in endemic areas and to prevent importation of the disease into, for example, the Pacific war zone, where a vector potential existed. The vaccine was prepared by the Rockefeller Foundation, according to specifications (*4*) that have remained essentially unchanged to the present day (*3*), as a lyophilized 10% suspension of viable YF virus from chick embryo cultures of the 17D strain. It was administered throughout the war in 0.5-ml doses subcutaneously, a method since shown to be superior to the multiple puncture (*5*) and scratch techniques (*3*). The only change in the preparation during the war occurred in June 1942, when serum-free vaccine was substituted for the original human-serum-stabilized material, following a global epidemic of 17D vaccine-related serum hepatitis (*4*).

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METHODS

Participants were recruited into the study if they had been on active duty between 1941 and 1945, and had not travelled since 1948 to areas where YF vaccination was required. Subjects were recruited from two Veterans' Administration (VA) hospitals in Colorado and Texas, and from Veterans of Foreign Wars (VFW) organizations in two Colorado cities. Recruitment was simpler and more effective through the VFW organizations, but the military service medical records of the hospitalized participants were more readily accessible, either at the hospital or at a regional records storage area.

A form was completed for each participant and included an identification code, the branch of service (army, air corps, or navy), dates and geographical areas of service, subject's recollection of vaccination (including YF vaccine), and history of travel since 1948 to areas where YF vaccine was required. Participants were not accepted for the study if they might have received YF vaccine since 1948.

A serum sample was obtained from each participant and tested for YF antibody by one or two test methods. Plaque-reduction neutralization tests (PRNT) against 17D YF virus were performed at the Vector-Borne Diseases Division (VBDD),[a] Centers for Disease Control. Mouse protection tests (by intracerebral inoculation in 6-week-old mice) against YF French neurotropic virus (FNV) were done at Yale Arbovirus Research Unit (YARU).[a]

Participants were assigned to the "vaccinated" group if they met one of the following criteria: (*a*) service in the army air corps between November 1942 and September 1945; (*b*) service in the Pacific theatre between September 1943 and June 1948; (*c*) service in the tropics of the western hemisphere between January 1941 and September 1945; (*d*) service in the US armed forces in any capacity between February and June 1942. These areas and times of service are those in which YF vaccine was administered to servicemen. Subjects not meeting any of these criteria were assigned to the "not vaccinated" group. Subsequent use of the terms "vaccinated" and "not vaccinated" in this report denotes this classification scheme and does not, except where otherwise stated, imply that the immunization status has been confirmed.

[a] A WHO Collaborating Centre for Arbovirus Reference and Research.

RESULTS

Serum samples from 149 servicemen were examined for antibody to YF virus by one or two test methods. The results of the PRNT and mouse-protection test are compared in Table 1. Of the 39 individuals without antibody by the PRNT (titre <2), 38 were also seronegative by the mouse-protection test. However, of the 48 persons with no detectable antibody by the mouse-protection test, only 38 were also negative (titre <2) by the PRNT, suggesting that the PRNT with 17D virus is more sensitive for detection of antibody.

Table 2 presents the PRNT results for the entire study population. Of the 33 non-vaccinated veterans, 27 (81.8%) had no detectable antibody while only 25 (21.6%) of 116 vaccinated veterans were seronegative. Vaccinated individuals were thus four times more likely to be seropositive to 17D YF virus than

Table 1. Correlation of mouse-protection test using French neurotropic yellow fever virus with PRNT to 17D yellow fever virus

Vaccination status	Mouse protection	PRNT titre							Total
		< 2	2	4	8	16	32	⩾ 64	
Vaccinated	none	19	1	4	2	0	1	2	29
	protection[a]	1	1	2	7	9	15	39	74
	total	20	2	6	9	9	16	41	103
Not vaccinated	none	19	0	0	0	0	0	0	19
	protection[a]	0	0	0	0	2	1	0	3
	total	19	0	0	0	2	1	0	22
All subjects	none	38	1	4	2	0	1	2	48
	protection[a]	1	1	2	7	11	16	39	77
	total	39	2	6	9	11	17	41	125

[a] Partial or complete protection.

Table 2. Prevalence of 17D yellow fever neutralizing (PRNT) antibody by immunization status on the basis of service history during the Second World War

PRNT titre	Vaccinated		Not vaccinated		Total
	No.	%	No.	%	
< 2	25	21.6	27	81.8	52
2	2	1.7	0	0	2
4	6	5.2	0	0	6
8	10	8.6	1	3.0	11
16	10	8.6	2	6.1	12
32	19	16.4	1	3.0	20
⩾ 64	44	37.9	2	6.1	46
Total	116	100.0	33	100.0	149

those who had presumably received no vaccine (78.4% and 18.2%, respectively). The results also showed that 92% of the 97 persons with detectable antibody had a titre ⩾ 8 and that 68% had a titre ⩾ 32.

In order to try to verify the subjects' YF vaccination status, we reviewed the medical records of the first 21 participants in this study. Service records were available for 18 subjects, of whom 15 were classified by service history criteria as vaccinated, and 3 as non-vaccinated. The medical records of only two veterans noted that YF vaccine had been administered; both subjects were in the vaccinated group, and antibody to 17D YF virus was demonstrated in both sera. It is noteworthy that, although the medical service record forms contained space to check the administration of other vaccines (e.g., smallpox, typhoid, typhus), there was no designated space where YF vaccination could be noted. Hence, recording the administration required an active, handwritten entry. The review of the first 21 study participants revealed that the medical records were of no value in establishing whether or not vaccine had been administered.

Sixteen participants claimed to recall specifically having received YF vaccine. Two of these, both seronegative, were classified by service history as non-vaccinated. Of the 14 classed as vaccinated, half were seropositive. One participant who had had hepatitis after the vaccination and two whose military records documented that YF vaccine had been administered were seropositive (one with a titre of 32 and two with titres of 64 or over).

Of 103 veterans[b] with service histories compatible with vaccination, 45 had served in the army, 36 in the navy, and 22 in the air corps. All of the vaccinated navy participants, 69% of the army, and 59% of the air corps subjects had served either in the Pacific or tropical America (criteria 2 and 3). Overall, 77% of the vaccinated participants had served in these zones. The remaining 23% were classed as vaccinated on the basis of service during a period when the service branches were immunizing all personnel against yellow fever (criteria 1 and 4).

Twenty of the 103 vaccinated veterans were found to be seronegative (Table 3). Of these, 90% were army personnel and 5% each were navy and air corps (one individual from each branch). The number of seronegative army veterans (18) is significantly greater than the 9 expected from the proportion of army veterans in the immunized study population ($P<0.01$).

There are two possible reasons for the presence of seronegative participants in the immunized group: (*a*) these individuals had been immunized but had reverted to seronegativity, or (*b*) they had not been immunized, either because the vaccine had been mishandled and so was not viable, or because they had inadvertently not received the vaccination. The latter possibility was probably more likely for the army than for either the navy or air corps, and is further suggested by an evaluation of the rates of seropositive immunized participants within each branch (Table 3). Of 45 army participants who had presumably received YF vaccine, 60% were seropositive; of 58 navy and air corps participants in the vaccinated group, 97% were seropositive.

We also considered the possibility that some personnel may have received more than one dose of YF vaccine. Two factors were evaluated: (*a*) the correlation between the mouse-protection test results and the branch of service among participants with detectable PRNT antibody, and (*b*) the correlation between high PRNT titre and branch of service. Of army personnel with a PRNT titre greater than 2, 85.2% also showed mouse-protection antibodies, compared with 90.7% of navy and air corps personnel; these data indicated no significant difference between the two groups. Similarly, there was no difference in the proportions of seropositive navy/air corps and army veterans with a high titre of PRNT antibody. Consequently, there is no evidence to confirm or refute the suggestion that the observed higher proportion of immunized navy and air corps veterans with detectable 17D antibody resulted from multiple immunizations.

Veterans were also compared on the basis of the number of criteria met for vaccination classification. Of the total vaccinated population, 64% met only one of the four criteria, 33% met two criteria, and 3% met 3 or 4 criteria. Because of the small number meeting three or more criteria, subjects were divided into those meeting only one criterion and those meeting two or more. Of veterans with only one qualifying criterion, 74% were seropositive (titre ⩾ 2), compared with 92% of veterans meeting two or more criteria ($P<0.05$).

[b] The branch of service of 13 participants was not available for this analysis.

Table 3. Yellow fever PRNT antibody titre in vaccinated Second World War veterans in 1975–76

Service branch	No. of subjects	Titre ⩾ 2		⩾ 4		⩾ 8		⩾ 16	
		No.	%	No.	%	No.	%	No.	%
Army	45	27	60	27	60	24	53	21	47
Navy and air corps[a]	58	56	97	54	93	51	88	45	78
Total	103	83	80.6	81	78.6	75	72.8	66	64.1

[a] When examined separately, the only substantial difference in the results for navy and air corps personnel occurred at the highest titres; specifically, 83% of navy participants had a titre ⩾ 16 compared with 68% of air corps participants.

Examination of Table 4 reveals no significant branch-of-service association with the number of criteria met (67% of army and 62% of other branches met only one criterion). Army veterans meeting one criterion had an antibody prevalence of 50% compared with 80% for those meeting two or more criteria. Of navy and air corps veterans, 94% of those who met only one criterion were seropositive, compared with 100% of those meeting two or more.

These results suggest that either (*a*) service in more than one qualifying area increased the chance that at least one viable vaccine dose was given, or (*b*) multiple qualifying service factors resulted in some veterans receiving YF vaccine more than once.

DISCUSSION

At the time of the first major public health application of YF immunization, i.e., among US troops during the Second World War, it was known that immunity following inoculation with 17D virus lasted a minimum of 4 years. This had been established by Fox & Cabral (*7*) who performed serial post-immunization neutralization tests on residents of the Brazilian highlands where YF was not endemic. Subsequent testing demonstrated continued immunity among these vaccinees 6 years after vaccination (*8*). Groot & Ribeiro (*9*) studied 108 people from the same group in 1958, 17 years after immunization, and found that 76% had readily demonstrable neutralizing antibody to the FNV strain of YF virus. Neutralization tests were performed in weanling mice using a single dilution of serum and virus; 21% were partially positive by this method (i.e., survival rates among the inoculated mice were $\frac{1}{5}-\frac{3}{5}$) and only 3% showed no protection. Of a control group of 78 unimmunized persons from the same area, only one had neutralizing antibody, indicating that natural flaviviral infection had not contributed to the high rate of seropositivity in the vaccinees. Rosenzweig et al. (*10*) investigated a group of 24 retired naval personnel who had been immunized 16–19 years earlier, and found that they all had neutralizing antibody. A constant serum-virus dilution neutralization test in suckling mice was used, and the mean log neutralization index was 4.2. These studies suggest that revaccination every 10 years is more frequent than necessary.

Table 4. Distribution of PRNT antibody titres among servicemen meeting one or more criteria for YF immunization

Branch	No. of criteria	Study group No.	% of branch	Prevalence of PRNT antibody (%) < 2	⩾ 2	⩾ 4	⩾ 8	⩾ 16	⩾ 32	⩾ 64
Army	1	30	67	50	50	50	47	43	33	23
	⩾ 2	15	33	20	80	80	67	53	53	33
Navy & air corps	1	36	62	6	94	89	86	75	67	50
	⩾ 2	22	38	0	100	100	91	82	68	55
Total	1	66	64	26	74	72	69	61	52	38
	⩾ 2	37	36	8	92	92	81	70	62	46

It is generally desirable to administer any prophylactic or therapeutic agent only as frequently, and only to as many people, as is necessary for the prevention or treatment of disease, and even a relatively safe vaccine should not be an exception to this principle. The results of the present study demonstrated that neutralizing antibody persisted for more than 30 years in 80.6% of veterans who had presumably been vaccinated. This lends credence to the opinion that the 17D YF attenuated strain may "confer an immunity which.. (is) almost life-long.." (*10*). Comparison of two subpopulations within this study revealed a marked difference in seropositivity 30 – 35 years after presumed vaccination; the army personnel had an antibody prevalence of 60% while the navy/air corps personnel were 97% seropositive. We have interpreted this to indicate that a significant proportion of army veterans either did not receive the vaccine or received improperly handled vaccine. Natural exposure to heterologous flaviviruses as a possible cause of persisting YF antibody would be expected to occur more frequently among army veterans than navy/air corps veterans, since the risk of exposure to vector-borne diseases for army personnel was at least equal to and more often greater than that for navy and air corps personnel.

These findings lead us to the following conclusions: (1) there is a high probability that PRNT antibody to 17D YF virus persists for 30 years or more following administration of a potent vaccine; (2) army personnel often failed to receive YF vaccine when they served in only one situation when vaccine should have been given; (3) some personnel were vaccinated, even though their service did not include vaccination-designated areas; and (4) 88% or more of veterans with persistent PRNT antibody to 17D virus also had mouse-protective antibodies against YF (FNV).

RÉSUMÉ

PERSISTANCE DE L'ANTICORPS NEUTRALISANT 30 À 35 ANS APRÈS VACCINATION PAR LE VACCIN ANTIAMARIL 17D

Des études antérieures sur la persistance de l'anticorps après vaccination par le virus vaccin antiamaril 17D (17D YF) ont montré que l'immunité persiste pendant 17 ans ou plus et peut-être pendant toute la vie. Nous avons étudié des sérums prélevés sur 149 anciens combattants de la deuxième guerre mondiale, 30 à 35 ans après leur service militaire au cours duquel la vaccination antiamarile était obligatoire pour des groupes définis. Du fait qu'il n'était pas possible d'obtenir de données sur la vaccination antiamarile à partir des dossiers médicaux des anciens combattants, on s'est fondé sur leurs états de service pour décider si ce vaccin avait été administré ou non. Il est probable que certains sujets dont les états de service indiquaient qu'ils auraient dû être vaccinés n'ont en fait jamais reçu le vaccin; c'est pourquoi la fréquence de l'anticorps dans ce groupe peut faire sous-estimer la persistance de l'immunité.

Les résultats de cette étude prouvent que l'anticorps neutralisant persiste pendant plus de trois décennies chez au moins 80% des anciens combattants qui ont probablement été vaccinés, et cela vient à l'appui de l'opinion selon laquelle la souche atténuée 17D YF confère une immunité pour la vie entière. Une proportion élevée (97%) des anciens combattants «vaccinés» de la marine et de l'aviation étaient séropositifs, alors que 60% seulement du personnel de l'armée de terre «vacciné» et 19% du personnel non vacciné des trois armes étaient séropositifs. Selon nous, la discordance marquée entre les chiffres relatifs aux anciens combattants vaccinés de l'armée de terre et ceux des deux autres armes indique qu'une proportion notable des premiers n'avaient pas reçu le vaccin ou bien avaient reçu un vaccin incorrectement manipulé. L'exposition naturelle à des flavivirus hétérologues, cause possible de la persistance des anticorps amarils, aurait, semble-t-il, dû se produire plus fréquemment parmi les anciens combattants de l'armée de terre que parmi ceux des deux autres armes, car le risque d'exposition à des maladies transmises par des vecteurs pendant des activités en temps de guerre était au moins égal et souvent plus grand pour le personnel de l'armée de terre que pour celui des deux autres armes. Ces résultats nous conduisent aux conclusions ci-après: 1) il y a une haute probabilité pour que l'anticorps neutralisant (réduction des plages) à l'égard du virus 17D YF persiste pendant 30 ans ou plus après administration d'un vaccin actif; 2) le personnel de l'armée de terre n'a souvent pas reçu de vaccin antiamaril lorsqu'il avait servi dans une situation seulement où ce vaccin aurait dû être donné; et 3) 88% ou plus des anciens combattants ayant un anticorps neutralisant (réduction des plages) à l'égard du virus 17D avaient également des anticorps protégeant la souris contre le virus neurotrope français.

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